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(12) **United States Patent
Simard**(10) **Patent No.: US 9,181,338 B2**(45) **Date of Patent: *Nov. 10, 2015**(54) **HUMAN ANTIBODY SPECIFIC FOR
IL-1ALPHA**(71) Applicant: **XBiotech, Inc.**, Vancouver (CA)(72) Inventor: **John Simard**, Austin, TX (US)(73) Assignee: **XBiotech, Inc.**, Vancouver (CA)

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This patent is subject to a terminal disclaimer.

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None

See application file for complete search history.

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XBiotech, Inc. Pressrelease: "XBiotech Files Investigational New Drug (IND) Application with the FDA for the treatment of Chronic Myelogenous Leukemia," Evaluate, Nov. 22, 2010.

Primary Examiner — Bridget E Bunner*Assistant Examiner* — Fozia Hamud(74) *Attorney, Agent, or Firm* — Stanley A. Kim(57) **ABSTRACT**Fully human monoclonal Abs includes (i) an antigen-binding variable region that exhibits very high binding affinity for IL-1 α and (ii) a constant region that is effective at both activating the complement system through C1q binding and binding to several different Fc receptors.**2 Claims, No Drawings**

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**HUMAN ANTIBODY SPECIFIC FOR
IL-1ALPHA****CROSS-REFERENCE TO RELATED
APPLICATIONS**

The present application is a continuation of U.S. nonprovisional patent application Ser. No. 14/177,892 filed on Feb. 11, 2014, now U.S. Pat. No. 8,956,831, which is a continuation application of U.S. nonprovisional patent application Ser. No. 13/762,453 filed on Feb. 8, 2013 (now U.S. Pat. No. 8,679,489), which is a continuation application of U.S. nonprovisional patent application Ser. No. 13/224,943 filed on Sep. 2, 2011 (now U.S. Pat. 8,388,956), which is a divisional application of U.S. nonprovisional patent application Ser. No. 12/455,458, filed on Jun. 1, 2009 (now U.S. Pat. No. 8,034,337), which claims the priority of U.S. provisional patent applications Ser. Nos. 61/057,586, 61/121,391, and 61/178,350 filed on May 30, 2008, Dec. 10, 2008, and May 14, 2009, respectively; all of which are incorporated herein by reference.

**STATEMENT AS TO FEDERALLY SPONSORED
RESEARCH**

Not applicable.

FIELD OF THE INVENTION

The invention relates generally to the fields of immunology, inflammation, cancer, vascular disorders, and medicine. More particularly, the invention relates to antibodies (Abs) which specifically bind interleukin-1 α (IL-1 α) and methods of using such Abs to treat, prevent, or detect a pathology associated with aberrant IL-1 α expression.

BACKGROUND

IL-1 α is pro-inflammatory cytokine that plays a role in a number of different activities including inflammation, immune responses, tumor metastasis, and hematopoiesis. IgG autoantibodies against IL-1 α occur naturally in the general human population and are thought to be beneficial in diseases such as atherosclerosis.

SUMMARY

The invention is based on the development of fully human monoclonal Abs (mAbs) that include (i) an antigen-binding variable region that exhibits very high binding affinity for human IL-1 α and (ii) a constant region that is effective at both activating the complement system through C1q binding and binding to several different Fc receptors. The IL-1 α specific mAbs described herein was made by replacing the constant region of a human IgG4 mAb having a variable region specific for human IL-1 α with the constant region of a human IgG1 mAb.

Accordingly, the invention features a purified human IgG1 mAb that specifically binds to human IL-1 α , the mAb including a heavy chain covalently joined to a light chain. The heavy chain can include the amino acid sequence of SEQ ID NO: 9 and the light chain can include the amino acid sequence of SEQ ID NO:11.

Also within the invention is a set of isolated nucleic acids including a first nucleic acid encoding the heavy chain of a human IgG1 mAb that specifically binds to IL-1 α , and a second nucleic acid encoding the light chain of the human

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IgG1 mAb that specifically binds to human IL-1 α . The first nucleic acid can encode the amino acid sequence of SEQ ID NO: 9 and the second nucleic acid can encode the amino acid sequence of SEQ ID NO:11. The first nucleic acid can include the nucleotide sequence of SEQ ID NO: 10 and the second nucleic acid can include the nucleotide sequence of SEQ ID NO:12.

In another aspect, the invention features an expression vector including a nucleic acid encoding the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

Another feature of the invention is an isolated host cell (e.g. a mammalian cell such as a CHO cell) including set of isolated nucleic acids including a first nucleic acid encoding the heavy chain of a human IgG1 mAb that specifically binds to IL-1 α , and a second nucleic acid encoding the light chain of the human IgG1 mAb that specifically binds to human IL-1 α . The heavy chain can include the amino acid sequence of SEQ ID NO: 9 and a light chain can include the amino acid sequence of SEQ ID NO:11.

The invention further features a method of killing a cell expressing human IL-1 α . This method can include the step of contacting the cell with a purified human IgG1 mAb that specifically binds to human IL-1 α .

A method of inhibiting migration of a human cell through a basement membrane matrix is also within the invention. This method can include the step of adding a purified mAb that specifically binds to human IL-1 α to a mixture including a basement membrane matrix and the human cell.

Further within the invention is a method of inhibiting an IL-1 α -induced increase in ICAM-1 and/or E-selectin expression on the surface of a human endothelial cell. This method can include the step of adding a purified mAb that specifically binds to human IL-1 α to a mixture including the endothelial cell and IL-1 α .

The invention additionally includes a method of tracking inflammation in a human subject previously subjected to the steps of: obtaining from the subject a first sample of peripheral blood mononuclear cells at a first time; contacting the first sample with a purified mAb that specifically binds to human IL-1 α ; and determining the percent of cells in the first sample that bind the monoclonal Ab. This method can include the steps of: (a) obtaining from the subject a second sample of peripheral blood mononuclear cells at a second time; (b) contacting the second sample with the purified mAb that specifically binds to human IL-1 α ; (c) determining the percent of cells in the second sample that bind the monoclonal Ab; and (d) comparing the percent of cells in the first sample that bind the mAb to the percent of cells in the second sample that bind the monoclonal Ab.

In the foregoing methods, the purified mAb can be a human IgG1 mAb including a heavy chain covalently joined to a light chain, e.g., wherein the heavy chain includes the amino acid sequence of SEQ ID NO: 9 and the light chain includes the amino acid sequence of SEQ ID NO:11.

Another method within the invention features the steps of: (a) enriching a biological sample obtained from a human subject using a filter to separate molecules according to molecular weight into a first fraction including intact IgG complexed with IL-1 α and second fraction including molecules less than 100 Kda; and (b) quantifying the amount of IL-1 α in the first fraction.

Yet another method within the invention features the steps of: (a) enriching a sample of plasma obtained from a human subject using a filter that separates molecules according to molecular weight into a first fraction including intact IgG complexed with IL-1 α and second fraction including molecule less than 100 Kda; (b) adding the first fraction to a

substrate including immobilized anti-human IgG Abs under conditions that allow IgG in the first fraction to specifically bind the anti-human IgG Abs immobilized on the substrate; (c) washing the substrate to remove material in the first fraction that does not specifically bind the immobilized anti-human IgG Abs; (d) contacting the substrate washed in step (c) with an Ab that specifically binds human IL-1 α under conditions that allows the Ab that specifically binds human IL-1 α to specifically bind any human IL-1 α bound to the substrate; (e) washing the substrate to remove any of the Ab that specifically binds human IL-1 α that is not bound to the substrate; and (f) quantifying the amount of Ab that specifically binds human IL-1 α remaining bound to the substrate after step (e).

Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Commonly understood definitions of biological terms can be found in Rieger et al., *Glossary of Genetics: Classical and Molecular*, 5th edition, Springer-Verlag: New York, 1991; and Lewin, *Genes V*, Oxford University Press: New York, 1994.

The term "specifically binds", as used herein, when referring to a polypeptide (including Abs) or receptor, refers to a binding reaction which is determinative of the presence of the protein or polypeptide or receptor in a heterogeneous population of proteins and other biologics. Thus, under designated conditions (e.g. immunoassay conditions in the case of an Ab), the specified ligand or Ab binds to its particular "target" and does not bind in a significant amount to other proteins present in the sample or to other proteins to which the ligand or Ab may come in contact in an organism. Generally, a first molecule that "specifically binds" a second molecule has an equilibrium affinity constant greater than about 10^5 (e.g., 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , and 10^{12} or more) liters/mole for that second molecule.

When referring to a protein molecule such as an Ab, "purified" means separated from components that naturally accompany such molecules. Typically, an Ab or protein is purified when it is at least about 10% (e.g., 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 99.9%, and 100%), by weight, free from the non-Ab proteins or other naturally-occurring organic molecules with which it is naturally associated. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. A chemically-synthesized protein or other recombinant protein produced in a cell type other than the cell type in which it naturally occurs is "purified."

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All applications and publications mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions will control. In addition, the particular embodiments discussed below are illustrative only and not intended to be limiting.

DETAILED DESCRIPTION

The invention encompasses compositions and methods relating to fully human mAbs that include (i) an antigen-binding variable region that exhibits very high binding affinity for IL-1 α and (ii) a constant region that is effective at both activating the complement system through C1q binding and binding to several different Fc receptors. The below described

preferred embodiments illustrate adaptation of these compositions and methods. Nonetheless, from the description of these embodiments, other aspects of the invention can be made and/or practiced based on the description provided below.

Methods involving conventional immunological and molecular biological techniques are described herein. Immunological methods (for example, assays for detection and localization of antigen-Ab complexes, immunoprecipitation, immunoblotting, and the like) are generally known in the art and described in methodology treatises such as *Current Protocols in Immunology*, Coligan et al., ed., John Wiley & Sons, New York. Techniques of molecular biology are described in detail in treatises such as *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Sambrook et al., ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and *Current Protocols in Molecular Biology*, Ausubel et al., ed., Greene Publishing and Wiley-Interscience, New York. Ab methods are described in *Handbook of Therapeutic Abs*, Dubel, S., ed., Wiley-VCH, 2007. Cell culture techniques are generally known in the art and are described in detail in methodology treatises such as *Culture of Animal Cells: A Manual of Basic Technique*, 4th edition, by R Ian Freshney, Wiley-Liss, Hoboken, N.J., 2000; and *General Techniques of Cell Culture*, by Maureen A Harrison and Ian F Rae, Cambridge University Press, Cambridge, UK, 1994. Methods of protein purification are discussed in *Guide to Protein Purification: Methods in Enzymology*, Vol. 182, Deutscher M P, ed., Academic Press, San Diego, Calif., 1990.

In one aspect, the invention features a fully human mAb that includes (i) an antigen-binding variable region that exhibits very high binding affinity for human IL-1 α and (ii) a constant region that is effective at both activating the complement system through C1q binding and binding to several different Fc receptors. The human Ab is preferably an IgG1. The K_a of the Ab is preferably at least $1 \times 10^9 \text{ M}^{-1}$ or greater (e.g., greater than $9 \times 10^{10} \text{ M}^{-1}$, $8 \times 10^{10} \text{ M}^{-1}$, $7 \times 10^{10} \text{ M}^{-1}$, $6 \times 10^{10} \text{ M}^{-1}$, $5 \times 10^{10} \text{ M}^{-1}$, $4 \times 10^{10} \text{ M}^{-1}$, $3 \times 10^{10} \text{ M}^{-1}$, $2 \times 10^{10} \text{ M}^{-1}$, or $1 \times 10^{10} \text{ M}^{-1}$).

Because B lymphocytes which express Ig specific for human IL-1 α occur naturally in human beings, a presently preferred method for raising mAbs is to first isolate such a B lymphocyte from a subject and then immortalize it so that it can be continuously replicated in culture. Subjects lacking large numbers of naturally occurring B lymphocytes which express Ig specific for human IL-1 α may be immunized with one or more human IL-1 α antigens to increase the number of such B lymphocytes. Human mAbs are prepared by immortalizing a human Ab secreting cell (e.g., a human plasma cell). See, e.g., U.S. Pat. No. 4,634,664.

In an exemplary method, one or more (e.g., 5, 10, 25, 50, 100, 1000, or more) human subjects (e.g., subjects not previously administered a human IL-1 α vaccine) are screened for the presence of such human IL-1 α -specific Ab in their blood. Those subjects that express the desired Ab can then be used as B lymphocyte donors. In one possible method, peripheral blood is obtained from a human donor that possesses B lymphocytes that express human IL-1 α -specific Ab. Such B lymphocytes are then isolated from the blood sample, e.g., by cells sorting (e.g., fluorescence activated cell sorting, "FACS"; or magnetic bead cell sorting) to select B lymphocytes expressing human IL-1 α -specific Ig. These cells can then be immortalized by viral transformation (e.g., using EBV) or by fusion to another immortalized cell such as a human myeloma according to known techniques. The B lymphocytes within this population that express Ig specific for human IL-1 α can then be isolated by limiting dilution meth-

ods (e.g., cells in wells of a microtiter plate that are positive for Ig specific for human IL-1 α are selected and subcultured, and the process repeated until a desired clonal line can be isolated). See, e.g., Goding, *Monoclonal Abs: Principles and Practice*, pp. 59-103, Academic Press, 1986. Those clonal cell lines that express Ig having at least nanomolar or picomolar binding affinities for human IL-1 α are preferred. MAbs secreted by these clonal cell lines can be purified from the culture medium or a bodily fluid (e.g., ascites) by conventional Ig purification procedures such as salt cuts, size exclusion, ion exchange separation, and affinity chromatography.

Although immortalized B lymphocytes might be used in vitro cultures to directly produce mAbs, in certain cases it might be desirable to use heterologous expression systems to produce mAbs. See, e.g., the methods described in U.S. patent application Ser. No. 11/754,899, now U.S. Pat. No. 8,524,865. For example, the genes encoding an mAb specific for human IL-1 α might be cloned and introduced into an expression vector (e.g., a plasmid-based expression vector) for expression in a heterologous host cell (e.g., CHO cells, COS cells, myeloma cells, and *E. coli* cells). Because Igs include heavy (H) and light (L) chains in an H₂L₂ configuration, the genes encoding each may be separately isolated and expressed in different vectors.

Although generally less preferred, chimeric mAbs (e.g., "humanized" mAbs), which are antigen-binding molecules having different portions derived from different animal species (e.g., variable region of a mouse Ig fused to the constant region of a human Ig), might be used in the invention. Such chimeric Abs can be prepared by methods known in the art. E. G., Morrison et al., *Proc. Nat'l. Acad. Sci. USA*, 81:6851, 1984; Neuberger et al., *Nature*, 312:604, 1984; Takeda et al., *Nature*, 314:452, 1984. Similarly, Abs can be humanized by methods known in the art. For example, monoclonal Abs with a desired binding specificity can be commercially humanized or as described in U.S. Pat. Nos. 5,693,762; 5,530,101; or 5,585,089.

The mAbs described herein might be affinity matured to enhance or otherwise alter their binding specificity by known methods such as VH and VL domain shuffling (Marks et al. *Bio/Technology* 10:779-783, 1992), random mutagenesis of the hypervariable regions (HVRs) and/or framework residues (Barbas et al. *Proc Nat. Acad. Sci. USA* 91:3809-3813, 1994; Schier et al. *Gene* 169:147-155, 1995; Yelton et al. *J. Immunol.* 155:1994-2004, 1995; Jackson et al., *J. Immunol.* 154 (7):3310-9, 1995; and Hawkins et al., *J. Mol. Biol.* 226:889-896, 1992. Amino acid sequence variants of an Ab may be prepared by introducing appropriate changes into the nucleotide sequence encoding the Ab. In addition, modifications to nucleic acid sequences encoding mAbs might be altered (e.g., without changing the amino acid sequence of the mAb) for enhancing production of the mAb in certain expression systems (e.g., intron elimination and/or codon optimization for a given expression system). The mAbs described herein can also be modified by conjugation to another protein (e.g., another mAb) or non-protein molecule. For example, a mAb might be conjugated to a water soluble polymer such as polyethylene glycol or a carbon nanotube (See, e.g., Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605, 2005). See, U.S. patent application Ser. No. 11/754,899.

Preferably, to ensure that high titers of human IL-1 α -specific mAb can be administered to a subject with minimal adverse effects, the mAb compositions of the invention are at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.9 or more percent by weight pure (excluding any excipients). The mAb compositions of the invention might include only a

single type of mAb (i.e., one produced from a single clonal B lymphocyte line) or might include a mixture of two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) different types of mAbs. In addition to human IL-1 α mAbs, the Ab compositions of the invention might also include other mAbs that specifically bind antigens other than human IL-1 α .

To modify or enhance their function, the human IL-1 α mAbs might be conjugated another molecule such as a cytotoxin or detectable label. A human IL-1 α specific mAb might be conjugated with one or more cytotoxins to more effectively kill cells expressing IL-1 α . Cytotoxins for use in the invention can be any cytotoxic agent (e.g., molecule that can kill a cell after contacting the cell) that can be conjugated to a human IL-1 α specific mAb. Examples of cytotoxins include, without limitation, radionuclides (e.g., ³⁵S, ¹⁴C, ³²P, ¹²⁵I, ¹³¹I, ⁹⁰Y, ⁸⁹Zr, ²⁰¹Tl, ¹⁸⁶Re, ¹⁸⁸Re, ⁵⁷Cu, ²¹³Bi, and ²¹¹At), conjugated radionuclides, and chemotherapeutic agents. Further examples of cytotoxins include, but are not limited to, antimetabolites (e.g., 5-fluorouracil (5-FU), methotrexate (MTX), fludarabine, etc.), anti-microtubule agents (e.g., vincristine, vinblastine, colchicine, taxanes (such as paclitaxel and docetaxel), etc.), alkylating agents (e.g., cyclophosphamide, melphalan, bischloroethylnitrosurea (BCNU), etc.), platinum agents (e.g., cisplatin (also termed cDDP), carboplatin, oxaliplatin, JM-216, CI-973, etc.), anthracyclines (e.g., doxorubicin, daunorubicin, etc.), antibiotic agents (e.g., mitomycin-C), topoisomerase inhibitors (e.g., etoposide, tenoposide, and camptothecins), or other cytotoxic agents such as ricin, diphtheria toxin (DT), *Pseudomonas* exotoxin (PE) A, PE40, abrin, saporin, pokeweed viral protein, ethidium bromide, glucocorticoid, anthrax toxin and others. See, e.g., U.S. Pat. No. 5,932,188.

The human IL-1 α specific mAb can also be conjugated to a detectable label. Useful detectable labels in the present invention include biotin or streptavidin, magnetic beads, fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, ³²P, ¹¹¹In, ⁹⁷Ru, ⁶⁷Ga, ⁶⁸Ga, or ⁷²As), radioopaque substances such as metals for radioimaging, paramagnetic agents for magnetic resonance imaging, enzymes (e.g., horseradish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters. Fluorescent markers may also be used and can be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

The present invention also encompasses nucleic acid molecules encoding fully human mAbs specific for human IL-1 α . Although the same nucleic acid molecule might encode both the heavy and light chains of a human IL-1 α -specific mAb, two different nucleic acid molecules, one encoding the heavy chain and the other encoding the light chain might also be used. The amino acid sequences of three IgG1 mAbs specific for human IL-1 α are presented herein. See SEQ ID NOs: 1, 3, 5, 7, 9, and 11. Exemplary nucleic acid molecules encoding these amino acid sequences are also described herein. See SEQ ID NOs: 2, 4, 6, 8, 10, and 12. Any other suitable nucleic acid that encodes the amino acid sequences of the two described IgG1 mAbs or other mAbs within the invention might also be used.

For production of mAbs, the nucleic acid molecules of the invention might be incorporated into an expression vector in an orientation wherein such nucleic acid molecules are operatively linked to expression control sequences such as transcriptional and translational control sequences. Examples of expression vectors include vectors derived from plasmids and vectors derived from viruses such as adenoviruses, adeno-associated viruses, and retroviruses. The nucleic acid molecules encoding a light chain and a heavy chain might be incorporated into a single vector or different vectors. The vectors of the invention might also include regulatory sequences such as promoters and/or enhancers (see, U.S. Pat. Nos. 5,168,062, 4,510,245 and 4,968,615), selectable markers, or sequences encoding affinity tags (for facilitating purification) or a detectable label.

For production of mAbs, the vectors of the invention can be introduced into a suitable host cell, e.g., a prokaryotic cell such as a bacteria or, preferably, a eukaryotic cell such as mammalian, plant, or yeast host cell. Examples of methods for introducing heterologous polynucleotides into host cells include use of viral vectors, electroporation, encapsulation of the polynucleotide(s) in liposomes, dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, Agrobacterium-mediated transformation, biolistic transformation, and direct microinjection of the DNA into nuclei. Mammalian cell lines are presently preferred for expression of mAbs from vectors. Examples of mammalian host cells include Chinese hamster ovary (CHO) cells (e.g., the DG44 CHO cell line), HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), NS0 cells, SP2 cells, HEK-293T cells, 293 Freestyle cells, and NIH-3T3 cells. The mAbs of the invention might also be expressed in transgenic animals or plants. See, e.g., U.S. Pat. Nos. 5,827,690; 5,756,687; 5,750,172; 5,741,957; 6,046,037; and 5,959,177.

The invention provides a method for detecting a human IL-1 α -expressing cell in a sample by contacting the cell with a human IL-1 α -specific mAb and detecting the mAb bound to the cell. The invention also provides a method for killing a human IL-1 α -expressing cell by contacting the cell with a human IL-1 α -specific mAb. Such killing can be accomplished by complement-mediated killing, Ab-dependent cell-mediated cytotoxicity, or Ab-mediated delivery of a cytotoxin. The Abs described herein have also been shown to be useful for other methods.

For example, MABp1 has been to reduce IL-1 α induced ICAM1 and E-selectin expression on endothelial cells. MABp1 has also been shown to be used in immunoassays for detecting and quantifying IL-1 α in a biological sample.

EXAMPLES

Example 1

Cloning of anti-hIL-1 α IgG1 and Kappa Chains

Variable region heavy chain (V-HC) and variable region light chain (V-LC) sequences were gene synthesized using amino acid sequence information provided in U.S. Pat. No. 5,959,085. V-HC was PCR amplified introducing HindIII/

corresponded to Swiss-Prot entry P01857, except for a K171Q and a V261L exchange. The V-HC and constant IgG1-HC sequence were ligated using the NheI site and cloned into pcDNA3 using HindIII and BamHI sites.

>hIL-1 α -IgG1-HC

(SEQ ID NO: 1)

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSRLRLSCTASGF

TFSMFGVHWVRQAPGKGLEWVAASVYDGSNKYYAESVKGRFTISR

NSKNILFLQMDSLRLLEDTAIVYVCARGRPKVVIPAPLAHWGQGLTVT

FSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS

GALTSGVHTFPAVLQSSGLYSLSVTVPSLSLTQTYICNVNHKP

SNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLPEPKPKDTLM

ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAQTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR

EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIALEWESNGQPENN

YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY

TQKSLSLSPGK

>hIL-1 α -IgG1-HC

(SEQ ID NO: 2)

atggaggttcgggctgagtggtgttctcgtggtgctcgtcgctggg

gcgtgcagtgccaggtgcagctggtggagagtggtgggtggcggtggt

gcagcctggcgggtctctgcgcctgtcttgactgcctccggtttt

accttttctatgtttggtgtgactgggtgcgccaggtcccgcca

agggactggaatgggtggcggcgtgagttacgacgggtccaaca

atattacgtgagagcgtgaaaggcagattcaccatcagcagagat

aattccaagaatattctgttctcgtcagatggacagctctgagactgg

aggacactgctgtgtactactgcgcctcgtggacgacctaaagtggt

catccccgccccctggcacattggggccagggaactctggtgacc

ttttctagcgtagcaccaggggcccatcggtatccccctggcacc

ctcctccaagagcacctctggggccacagcgccctgggtgacctg

gtcaaggactacttccccgaacgggtgacgggtgctggtgaactcag

gcgccttgaccagcggtccacaccttccccggtgtctctacagtc

ctcaggactctactcctcagcagcgtagtgaacctgacctccagc

agcttgggcacccagacctacatctgcaacgtgaatcacaagccca

gcaacaccaaggtggacaagaaagttagagcccaaatctgtgacaa

aactcacacatgccaccgtgccagcacctgaactcctgggggga

ccgtcagttctctcttccccccaaaacccaaggacacctcatga

tctcccggaacctgaggtcacatgcgtggtggtggacgtgagcca

cgaagacctgaggtcaagttcaactggtacgtggacggcgtggag

gtgcataatgccagacaaagccgctggaggagcagtagacaacagca

cgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct

gaatggcaaggagtacaagtgaaggtctccaacaagacctccca

gcccccatcgagaaaacctctccaagccaaagggcagccccgag

aaccacaggtgtacacctgcccccatcccggtgagctgaccaa

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gaaccagggtcagcctgacctgctggtcaaaggttctatcccagc
 gacatcgccctggagtgaggagagcaatgggcagccggagaacaact
 acaagaccagcctcccgctgctggactccgacggctccttcttctc
 ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaac
 gtcttctcatgctccgtgatgcatgaggtctgcacaaccactaca
 cgcagaagagcctctccttaagtcgggaaaataa

The V-LC was PCR amplified introducing HindIII/ClaI sites upstream of the ATG start codon and a BsiWI site at the 3' end. The human constant Kappa-LC sequence was PCR amplified introducing a 5' BsiWI site encoding an additional Arg and the first amino acids Thr, and a BamHI site at the 3' end. The human constant Kappa-LC amino acid sequence corresponded to Swiss-Prot entry P01834. V-HC and constant Kappa-LC sequences were ligated using the BsiWI site and cloned into pcDNA3 using HindIII and BamHI sites.

>hIL-1a-K-LC

[SEQ ID NO: 3]
 MDMRVPQQLLGLLLWFPGRCDIQMTQSPSSVSASVGDRTITCR
 ASQGISSSLAWYQQKPGKAPKLLIYEASNLETGVPSRFSGSGSGSD
 FTLTISSLQPEDFATYYCQQTSSSELLSEGGGTKVEHRTVAAPSVFI
 FPPSDEQLKSGTASVCLNNFYFPREKQVQKVDNALQSGNSQESV
 TEQDSKDYSLSTLTLSKADYKHKVYACEVTHQGLSSPVTKSF
 NRGEC

>hIL-1a-K-LC

[SEQ ID NO: 4]
 atggacatgcgcgtgccccccagctgctggggtgctgctgctgt
 ggttcctcgatctaggtgcgacattcagatgacccagtcctccag
 ctgagtgacagctccgtggcgacagagtgacaatcacctgccgc
 gcctctcaggaatctctagttgctggcctggtaccagcagaagc
 ctggaagggccccaagctgctgatctatgaagcctccaacctgga
 gaccggcgtgcctctcgcttcagcggctcaggtcaggcagtgat
 ttactctgaccatcagctccctgcagccagaggatttcgctactt
 actactgccagcagacctcttcatctctgctgctcctcgggggaggc
 acaaagggtggagcaccgtacgggtggctgcacctctgtctcatct
 tcccccatctgatgagcagttgaaatctggaactgcctctgttgt
 gtgctgtgtaataacttctatcccagagaggccaaagtacagtggt
 aagggtggataaacgccctccaatcggttaactccaggagagtgta
 cagagcaggacagcaaggacagcacctacagcctcagcagcacct
 gacgtgagcaaaagcagactacgagaacacaaagtctacgcctgc
 gaagtacccatcagggcctgagttcacgggtgacaaagagcttca
 acaggggagagtgtag

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Example 2

Generation of NATHMAB-hIL-1 α IgG1 and Kappa Chain

The complete sequence encoding the NATHMAB-hIL-1 α /IgG1 heavy chain was gene synthesized. The V-HC sequence corresponded to the amino acid sequence described in U.S. Pat. No. 5,959,085. The human constant IgG1-HC sequence corresponded to Swiss-Prot entry P01857. The nucleotide sequence was codon optimized for expression in CHO cells. A Kozac sequence (gccacc) was added upstream of the start ATG.

>NATHMAB-hIL-1A-IGG1-HC

[SEQ ID NO: 5]
 MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGKSLRLSCTASGF
 TFSMFVHVRQAPGKGLEWVAASVDGSNKYYAESVKGRFTISR
 NSKNILFLQMDSLRLLEDTAIVYCCARGPRKPVVIPAPLAHWGQGLVT
 FSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNS
 GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKP
 SNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLM
 ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
 YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHY
 TQKSLSLSPGK

>NATHMAB-hIL-1A-IGG1-HC

[SEQ ID NO: 6]
 gccaccatggagtttggtctgtcctgggtgtctctgggtggctctgc
 tgaggggggtgcagtgccaggtccagctggtggagctctggtggggg
 agtgggtgcagcctgggagatctctcggtgtcttgcaactgcctct
 ggtttcactttctctatgtttgggtgtgcatgggtcaggaagcac
 caggcaaaggactcgagtggtgcagctgtgagctatgacgggtc
 taacaaatatcagctgagctctgtcaagggtagggttaccatcagc
 cgggataattccaaaatatcctgttctgcaaatggactctctga
 ggctggaagatactgcagctctactattgtgcaagggggaggccaaa
 ggtggtgatccccgctccccctcgctcactggggacagggaacctg
 gtgactttcagctctgctagcacaaggccctagcgtgttcccat
 tggctccttctccaaatctactcttgaggaccgccgccccctggg
 atgtctcgtaagattattttctgagcccgctaccgtgagctgg
 aacagcggcgccctgactagcggcgtgcacaccttcccgagctgc
 tgcaatctagcgggtgtactccctgagctctgtcgtgacgtgccc
 ctccagcagcctcggaactcagacctacatctgcaatgcaatcat
 aaacctctataacaaagtcgataagaaggtcgaaacctaatctt
 gcgataaaaccataacctgcccccttgcccagcaccggaactgct
 gggcggtccctctgtgtttctgttccccccaaacccaaagatacc
 ctgatgatctctaggacccccaggtcacttgtgtcgtggtggatg

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tgtccacgaagatccagaagtcaaattcaactggtatgtggacgg
 ggtcgaagtgcacaacgcaagaccaagcctagggaggaacagtat
 aatagcacatatagggtggtcagcgtcctgacgctcctgcacagc
 actggctgaatggcaagaatatagtgtaaagtgtccaacaaggc
 cctgccagcccaatcgaaaagacaatctctaaagccaaggggcaa
 ccccggaacctcaggtctatactgccaccctctcggtatgaac
 tgaccaagaatcaggtgagcctgacatgtcttgtaaggggttttta
 tccctccgacattgccgtggagtgaggagcaatggacaaccagaa
 aataactacaaaaccacccccctgtgctggactccgatggttct
 tcttctctactctaagctgacagtgagataagctcagtgaggcagca
 ggggaatgtgttctctgtctgtgatgcacgagcactgcacaat
 cattatacacaaaagtctctgtctctgtctccaggaaagtaa

The complete sequence encoding the NATHMAB-hIL-1 α /Kappa light chain was gene synthesized. The V-LC sequence corresponded to the amino acid sequence described in U.S. Pat. No. 5,959,085. The human constant Kappa-LC sequence corresponded to Swiss-Prot entry P01834. The nucleotide sequence was codon optimized for expression in CHO cells. A Kozac sequence (gccacc) was added upstream of ATG.

>NATHMAB-hIL-1A-K-LC

[SEQ ID NO: 7]

MDMRVPAQLLGLLLWFPGRCDIQMTQSPSSVSASVGRVTITCR
 ASQGISSWLAWYQQKPKAPKLLIYEASNLETGVPSRFSGSGSGSD
 FTLTISSLQPEDFATYYCQQTSSSELLSEGGGTKVEHTVAAPSVFIF
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
 EQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
 RGEC

NATHMAB-hIL-1A-K-LC

[SEQ ID NO: 8]

gccaccatggacatgcgcgttctgccagctcctcgactgctgc
 tgcttgggttccaggtcccggtgtgatattcagatgacacagtc
 tccctcctcgtatctgcacgtggcgacagggtcacaatcact
 ttagggccagccagggatctctagttggtcgcagtggtaccaac
 aaaagccaggttaaggtccgaaactgctcatttacgaagctagtaa
 cctcgaaacaggcgtgccaagccggttttagcggtccggtccgggt
 tctgacttcacctcactatttctcctccctgcaacctgaggattttg
 ccacatactactgtcagcaaaactcttcttttctgtctcctttgg
 tgggggaactaaggtggagcacacagtgccgcgccccagcgtcttt
 atcttcccccaagcgatgaacagctgaagtcagggaccgcccagcg
 tggctctgctgtcacaataattttaccctcgcgaggttaaggtcca
 atggaaagtgtataacgcctccagagcggttaactctcaggagctct
 gtcacagagcaagacagcaaggatagcacctattcctctccagca

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ccctgacactgtctaaagccgactacgagaaacacaaagtgtacgc
 ttgtgaggtgactcaccagggtgactgagtagcctgtgacaaaaatct
 tccaataggggagaatgctga

Example 3

Expression of NATHMAB-IL-1 α (IgG1/k Subtype)

NATHMAB-IL-1 α was expressed and purified using a transient transfection method. Cell culture supernatant or protein G affinity purified Ab was subjected to further analysis as described below. Human embryonic kidney (HEK) 293T cells were cultured in DMEM containing 10% FCS, and transiently transfected using jetPEI reagent (Polyplus) according to manufacturer's protocol. Cells were seeded on 10 cm dishes (3×10^6 cells per 10 cm dish) 24 h prior to transfection to reach approximately 50% density at the time point of transfection. 5 μ g per dish of pcDNA3-anti-hIL-1 α -IgG1-HC and a 2-fold molar excess of pcDNA3-anti-hIL-1 α -Kappa were used for transfection. After recovery, medium was changed to DMEM containing 2% FCS (10 ml per dish) and Ab was collected for 5 to 6 days. The supernatant was collected, filtered, pH adjusted to 7.5-8, and stored at 4° C. until further use.

Part of the supernatant (250 ml) was incubated with protein G sepharose (GE Healthcare) for 3 h at 4° C. on a rotation wheel. Then, the protein G sepharose was loaded onto a gravity flow column and washed with PBS. Ab was eluted in 1 ml fractions using 100 mM glycine/150 mM NaCl into 100 μ l Tris (pH 8), followed by dialysis with PBS containing 10% glycerol. The total protein concentration of each fraction was measured using the BCA Protein Detection Kit (Pierce). Correct size of heavy and light chains, and of the assembled native Ab was confirmed by SDS-PAGE.

Supernatant containing NATHMAB-hIL-1 α purified Ab and Triton X-100 cell lysates of producer HEK 293T cells were tested for antigen binding in a radioimmunoassay (RIA) using 125 I-hIL-1 α . Binding was assayed by absorption to protein G. All samples bound 125 I-hIL-1 α with highest activity in the eluate. Binding of purified NATHMAB-hIL-1 α in a concentration of 0.012% (half-max activity in RIA) to 125 I-hIL-1 α was used for measuring the affinity coefficient. The K_a of NATHMAB-hIL-1 α under these conditions was 3.03×10^{10} M $^{-1}$. Back calculation revealed an estimated concentration of approximately 30 μ g/ml active anti-hIL-1 α -IgG in the purified eluate.

Neutralizing activity of NATHMAB-hIL-1 α was tested in a bioassay using the murine EL4-6.1 subline which produces high levels of IL-2 when treated with murine or human IL-1 α (Zubler et al., J. Immunol. 134:3662-3668, 1985). The indicated concentrations of NATHMAB-hIL-1 α (eluate) were incubated for 30 min at 37° C. with various concentrations of recombinant hIL-1 α (eBioscience) in a final volume of 100 μ l/well in a 96-well culture plate (flat bottomed). Each point was carried out in triplicate and in culture medium (DMEM, 5% FCS). To each well were added 100 μ l of a suspension of EL4-6.1 cells (5×10^5 cells/ml) in culture medium containing 0.2 μ g/ml ionomycin. After incubation for 24 h at 37° C. in a 5% CO $_2$ incubator, cell free supernatants were harvested and assayed for IL-2 concentrations using a commercially available ELISA (R&D Systems). The results showed that NATHMAB-IL-1 α effectively neutralized hIL-1 α -induced IL-2 secretion by EL-4 cells.

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To test for neutralization of membrane-bound hIL-1 α , the same EL-4 cell-based assay as described above was used with following modifications. Different concentrations of NATHMAB-hIL-1 α (eluate) were incubated with various numbers of human activated monocytes. For monocyte preparation, PBMC were isolated from buffy coat using Ficoll-Paque centrifugation. Monocytes were allowed to adhere for 1.5 h at 37° C. in RPMI on plastic dishes. Non-adherent lymphocytes were washed away to yield a nearly pure monocyte culture. Monocytes were cultured in RPMI containing Gln, Pyr, and 10% FCS for 24 h with LPS (1 μ g/ml) at 37° C. in a 5% CO₂ incubator. Cells were detached with PBS/2 mM EDTA, carefully scraped from plates, and transferred into Falcon tubes. Cells were washed twice with PBS, resuspended in PBS/1% PFA and fixed for 10 min at 20° C. Cells were washed with glycine buffer (150 mM glycine, 75 mM NaCl, pH 7.4), then with culture medium and counted. The results showed that NATHMAB-hIL-1 α effectively neutralized IL-2 secretion by EL-4 cells induced by membrane-bound hIL-1 α . In an experiment similar to that described above, NATHMAB-hIL-1 α was tested for neutralization of murine IL-1 α . Indicated amounts of NATHMAB-hIL-1 α supernatant were incubated with recombinant human (h) or murine (m) IL-1 α (eBioscience). The supernatant containing the Ab neutralized human, but not murine, IL-1 α .

Example 4

Ab-Mediated Killing of Cancer Cells

Human peripheral blood mononuclear cells (PBMC) isolated from the buffy coat by standard Ficoll Paque preparation were incubated in either RPMI-1640 CM or RPMI-1640-CM containing rhIL-2 (30 ng/ml, ebioscience) at 37° C. and 5% CO₂ overnight and used as effector cells (E). THP1 cells were used as the targets (T). The assay was carried out in 96-well plates with each point in triplicate. After 1 \times 10⁴ targets that were incubated with different concentration of MABp1 for 15 mins, effector cells were added in an ET ratio of 25:1 and 50:1 to 1 \times 10⁴ targets and incubated for another 4 hours. 75 μ l of assay volume were transferred to a new 96-well plate and cytotoxicity was assayed using the LDH cytotoxicity detection kit (Roche) according to manufacturer's protocol. % specific lysis=(mean experimental release-mean spontaneous release without antibody) \times 100/(mean maximal release from targets-mean spontaneous release from targets) A. untreated PBMC were used as effector cells. B. rhIL-2-treated PBMC were used as effector cells. In both cases, increasing concentrations (1.25 to 20 μ g/ml) of MABp1 resulted in increased target cell killing (up to about 90%) at both ET ratios.

Example 5

Human Anti-IL1 α Specific mAb Sequences

The complete sequence encoding for another human anti-hIL-1 α IgG₁/Kappa light chain specific for human IL1 α (MABp1) was synthesized and expressed as described above. In the nucleic acids encoding the heavy and light chains, a Kozac sequence (gccacc) was added upstream of the start ATG.

Heavy Chain

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSRLSCTASGF

TFSMFGVHWVRQAPGKGLEWVAAVSYDGSNKYYAESVKGRFTISR

[SEQ ID NO: 9]

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NSKNILFLQMSLRLEDTAVYYCARGRPKVVIPAPLAHWGQGLTVT
FSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
SNTKVDKRVPEPKSCDKHTHTCPPCPAPELLGGPSVFLPEPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHY
TQKSLSLSPGK

[SEQ ID NO: 10]

gccaccatggagtttggctgtcctgggtgtctctgggtggctctgc
tgaggggggtgcagtgccaggtccagctggtaggtctggtagggg
agtgggtgcagcctgggagatctctcggctgtcttgactgcctct
gggttctactttctctatgtttgggtgtgcaagggtcaggcaagcacc
aggcaaggactcgagtggtgcagctgtgagctatgacgggtct
aacaaatattacgtgagctctgtcaagggttaggataccatcagccg
ggataattccaaaaatctcctgacctgcaaatggactctctgaggc
tggaagatactgcagctctactatgtgcaagggggaggccaaagg
ggtagtccccgctccccctcgctcactggggacagggaaccctggg
actttcagctctgctagcaccaggcccttagcgtgttccattgg
ctccacctccaaatctactctcggaggcaccgccctgggtagt
ctcgtgaaagattattttctcagccgtcaccgtgagctggaaca
gcggcgccctgactagcggcgtgcacaccatcccgagtgctgcaa
tctagcgggctgtactccctgagctctgtcgtgacccgtgccctcca
gcagcctcggaaactcagacctacatctgcaatgtcaatcataaacc
ctctaataccaaagtcgataaagggtcgaacctaaatcttgcgat
aaaaccatacctgcccccttgccagcaccgcgaactgctgggcg
gtccctctgtgatctgaccccccaaacccaaagatccctgatga
tctctaggacccccgaggtcactgtgtcgtgggtggatgtgtccca
cgaagatccagaagtc aaattcaactggtatgtggacggggtcgaa
gtgcacaacgcaaagaccaagcctaggagggaacagtataatagca
catatagggtggtcagcgtcctgaccgtcctgcatcaggactggct
gaatggcaagaatataagtgtaaagtgtccaacaaggccctgcc
gccccaatcgaaaagacaatctctaagccaaggggcaaccccg
aacctcaggtctatacactgccacctctcgaggaggaaatgaccaa
gaatcaggtgagcctgacatgtcagtgaaaggtattatccctccga
cattgccgtggagtgaggagcaatggacaaccagaaaataactac
aaaaccacacccctgtgctggactccgtaggacctctctctctcta
ctctaagctgacagtggataagtctagggtggcagcagggggaatgtg
actcctgctctgtgatgcacgaggcactgcacaatcattatacaca
aaagtctctgtctctgtctccaggaaagtaa

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Light Chain

[SEQ ID NO: 11]

MDMRVPAQLLGLLLWFPGSRCDIQMTQSPSSVSASVGDRTITCR

ASQGISSWLAWYQQKPGKAPKLLIYEASNLETVPSRFSGSGSGSD

FTLTISLQPEDFATYYCQQTSSSELLSEGGGKVEHKRTVAAPSVF

IFPPSDEQLKSGTASVVCLLNNFYPRKAKVQWKVDNALQSGNSQES

VTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS

FNRGEC

[SEQ ID NO: 12]

gccaccatggacatgcgcgttcctgccagctcctcgactgctgc

tgctaggaccaggctcccggtgtgatattcagatgacacagtctc

cctcctccgtatctgcatccgtggcgacagggtcacatcacttg

tagggccagccagggtatctctagaggctcgcatggtaccaacaaa

agccaggtaaggctccgaaactgctcatttacgaagctagtaacct

cgaaacaggcgtgccaagccgatagcggctccggaccggactgac

ttcacctcactatacctcctgcacactgaggattagccacatat

tactgtcagcaaaacttatcattctgctctcctaggtggaggaacta

agggtggagcacaagcggacagagctgctcctagcgtattatcaccc

tccaagcgatgaacagctgaagtccaggaccgccagcgtggtctgc

ctgctcaataattataccctcgcgaggctaaggctccaatggaagt

ggataacgcctccagagcggtaactctcaggagctgtgcacagag

caagacagcaaggatagcacctattccctctccagcacccctgacac

tgtctaaggccgactacgagaacacaaagtgtacgcttgtagggt

gactcaccagggtgagtagccctgtgacaaaatctttcaatagg

ggagaatgctga

Example 6

MABp1 Binding Affinity

The binding affinity of purified MABp1 was determined using surface plasmon resonance (SPR) on a BIAcore 2000 instrument (GE Health Sciences). A mouse monoclonal anti-human IgG (Fc) Ab was covalently immobilized on the flow cells of a CM5 sensor chip using a human Ab capture kit and amine coupling kit (GE Health Sciences). Immobilization levels of 8000-14000 RU would typically be achieved. After immobilization of the mouse anti-human IgG (Fc) capture Ab, three start-up cycles with HBS-EP running buffer (GE Health Sciences) and two start-up cycles with MABp1 were run to stabilize the CM5 surface and to remove any non-covalently bound Ab. For analysis, MABp1 Ab was diluted into HBS-EP running buffer to a final concentration of 1 μ g/mL and immobilized to 700 RU on one flow cell of the CM5 sensor chip. Carrier-free human IL-1A cytokine (eBioscience, #34-8019) was serially diluted in HBS-EP running buffer over a test range from 100 nM to 0.05 nM. Flow rate was 30 μ L/min. Dissociation data for each cytokine dilution was recorded for 15 minutes. The CM5 surface was regenerated after each cycle using a single injection of 3 M $MgCl_2$ for 25 seconds at a flow rate of 30 μ L/min. BiaEvaluation software and a Langmuir binding model was used to fit the data. The KD for MABp1 was determined to be less than 2.0×10^{-10} M.

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Example 7

MABp1 Inhibits Tumor Cell Invasion of a Basement Membrane Matrix

Matrigel (BD), a basement membrane matrix, was thawed at 4° C. overnight and the dilute (5 mg/ml to 1 mg/ml) in serum free cold cell culture media. 100 μ L of the diluted matrigel was placed into the upper chambers of a 24-well transwell (Costar) and the transwell was incubated at 37° C. for at least 4 to 5 h for gelling. Tumor cells (MDA-MB-231 and THP-1) were harvested from tissue culture flasks by Trypsin/EDTA, washed with culture media, and resuspended in medium containing 1% FBS at a density of 1×10^6 cells/ml. The gelled matrigel was gently washed with warmed serum free-culture media, and 100 μ L of the cell suspension was added in each well. The lower chamber of the transwell was filled with 600 μ L of culture media, and the plates were incubated at 37° C. for 12 to 24 h. The cells that did not invade the matrigel were gently scraped off the top of each transwell with a cotton swab. The transwells were then removed from the 24-well plates and stained with crystal violet after fixing the invaded cells with 70% ethanol or methanol. The invaded cells were counted under a light microscope. The percent of cells invading the matrigel was significantly inhibited in the presence of MABp1.

Example 8

MABp1 Blocks Increase in ICAM1 Expression in Endothelial Cells

Human umbilical vein endothelial cells (HUVEC) (BD Biosciences) were seeded to 24-well plates at 5×10^5 per well in 1 mL of M-200 medium supplemented with low-serum growth supplement (Invitrogen). Cells were allowed to settle for 3-4 hours. Medium was aspirated and a fresh 1 mL of M-200 was added per well. MABp1 was added directly to cells @ 4.26 μ g/mL, co-incubated for 15 minutes at room temperature, and then recombinant human IL-1 α (rhIL1A, eBioscience) was added to a final concentration of 40 pg/mL. Positive control wells received the addition of IL-1 α only. HUVEC cells in the absence of IL-1 α or the absence of MABp1 served as negative controls. After 17-20 hours incubation at 37° C., 5% CO_2 , cells were lifted from the plates by a non-enzymatic treatment for 20 minutes using CellStripper reagent (Cellgro Mediatech) and then immediately assayed for CD54 (ICAM-1) expression using standard flow cytometry protocols. Staining buffer comprised Dulbecco's PBS supplemented with 2% heat-inactivated fetal bovine serum. PE-conjugated mouse anti-human CD54 (ICAM-1) mAb (eBioscience, clone HA58) or a PE-conjugated mouse IgG1k isotype control (eBioscience, #12-4714) were used per manufacturer's instructions to stain HUVEC cells in a 100 μ L staining volume for 20 minutes in the dark at room temperature. Two washes in staining buffer were subsequently performed and then samples were acquired on a FAC-SCalibur flow cytometer (BD Biosciences). Among several independent experiments (n=5) the upregulation of ICAM-1 adhesion molecules induced by rhIL1A on the surface of HUVEC cells was neutralized by MABp1 to baseline levels exhibited by the unstimulated HUVEC cells.

Example 9

MABp1 Blocks Increase in E-selectin Expression in Endothelial Cells

Similar to its effects on ICAM-1 induction, MABp1-mediated neutralization of induction of CD62E (E-selectin) on

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HUVEC cells was also observed. This effect was most pronounced when HUVEC cells were stimulated not by soluble rhIL-1 α but by membranous IL-1 α anchored by glycosylphosphatidylinositol to the surface of DG44 CHO cells (GPI-IL1A cells). In this experiment, confluent cultures of HUVEC cells in 6-well plates were co-cultured overnight with 5 \times 10⁶ GPI-IL1A DG44 cells in M-200 medium, either alone, in the presence of 10 μ g/mL MABp1, or in the presence of 10 μ g/mL D5 isotype control Ab. After 17-20 hours, HUVEC monolayers were washed extensively with Dulbecco's PBS and then lifted by non-enzymatic treatment for 20 minutes with CellStripper reagent (Cellgro Mediatech) and then immediately assayed for CD62E (E-selectin) expression using standard flow-cytometry protocols. Staining buffer comprised Dulbecco's PBS supplemented with 2% heat-inactivated fetal bovine serum. PE-conjugated mouse anti-human CD62E mAb (eBioscience, clone P2H3) or a PE-conjugated mouse IgG1k isotype control (eBioscience, clone P3) were used per manufacturer's instructions to stain HUVEC cells in a 100 microliter staining volume for 20 minutes in the dark at room temperature. Two washes in staining buffer were subsequently performed and then samples were acquired on a FACSCalibur flow cytometer (BD Biosciences). Upregulated E-selectin expression on the surface of HUVEC cells induced by membranous GPI-IL-1 α was neutralized by MABp1 to baseline levels exhibited by unstimulated HUVEC cells.

Example 10

MRC-5 Bioassay for MABp1 Potency (Neutralization of rhIL1A)

The MRC-5 cell line, derived from fetal human lung fibroblasts, was obtained from the ATCC collection (CCL-171). The IL-1 neutralizing potency of MABp1 was assayed by measuring IL-1A induced release of IL-6 from MRC-5 cells. MRC-5 cells were seeded at 5 \times 10³ per well to a 96-well plate in 100 microliters of DMEM complete medium. Cells were cultured overnight at 37° C. in a humidified 5% CO₂ incubator. Confluent MRC-5 cells were subsequently cultured another 24 hours with 20 pg/mL of recombinant human IL-1A (rhIL1A, eBioscience) either alone or in the presence of increasing concentrations of MABp1. Negative control cells were not stimulated with rhIL1A. After the 24 hours, supernatants were collected and assayed for IL-6 release using and IL-6 ELISA kit from eBioscience. The IC₅₀, or concentration of MABp1 required to inhibit 50% of the maximal IL-6 release, was in the range of 0.001-0.01 μ g/mL.

Example 11

MABp1 Identifies IL-1 α + Cells

One hundred microliters of sodium heparin anti-coagulated whole blood was aliquoted to polystyrene FACS tubes. Samples were incubated at room temperature for 15 minutes with 1 mg of human IgG (protein-A purified) plus 2 ml of heat-inactivated fetal bovine serum to block Fc receptors. Primary Abs were then added to the sample: Either 1 mg of Alexa-488 labeled MABp1, 1 mg of FITC-labeled monoclonal anti-membrane human IL1A Ab (FAB200F, R&D Systems), or 1 mg of a murine isotype control (IC002F, R&D Systems). Primary Abs were incubated with sample for 30 minutes at room temperature in the dark. Sample erythrocytes were then lysed (BD Biosciences PharmLyse solution) at room temperature for 15 minutes, centrifuged at 300 \times g for 5 minutes, and aspirated. Sample pellets were washed three

18

times with 1 mL Hank's balanced salt solution (HBSS) containing 2% heat-inactivated fetal bovine serum. Sample was resuspended in 0.3 mL HBSS+2% FBS and data was acquired on a FACSCalibur flow cytometer and analyzed using CellQuest software. Flow cytometric analysis of human PBMC using MABp1 showed that only 0.2% of PBMC were positive for IL-1 α .

Example 12

MABp1 for Detecting and Tracking Infections and Inflammation

Flow cytometric analysis (as in Example 11) of human PBMC using MABp1 showed a 3.6-fold increase in the percent of PBMC positive for IL-1 α + in a subject with a sub-clinical infection compared to a normal control. Similarly, in a subject with an inflamed wisdom tooth, an increase in the percent of PBMC positive for IL-1 α + . A substantial decrease in the number of IL-1 α + PBMC was observed from 14 to 45 days after removal of the wisdom tooth.

Example 13

Immunoassay for Detecting and/or Quantifying IL-1 α

In general, very low levels of IL-1 α are present in the plasma of human subjects. Because these levels are often beyond the detection threshold of conventional immunoassays, an ELISA with improved sensitivity was developed. In this ELISA, exogenous anti-IL-1 α Ab (e.g., MABp1) can be added to a biological sample being tested (e.g., human plasma) under conditions that allow the Ab to bind IL-1 α in the sample. Because, it was observed that almost all IL-1 α in human plasma samples exists already bound to endogenous anti-IL-1 α Ab, the latter step can often be omitted. The sample with IL-1 α -Ab complexes is then applied to a filter (Amicon centrifugal device) with a molecular weight cutoff of about 100 kDa to separate the IL-1 α -Ab complexes from molecules in the sample less than the molecular weight cutoff. In one experiment, this resulted in a 50-fold concentration. The processed sample (and dilutions thereof) was then added to wells of a microtiter plate coated with an anti-human IgG capture Ab (2 μ g/ml mouse anti-human IgG, Fc-specific, Southern Biotech product code #9042-01). After allowing time to bind the IL-1 α -Ab complexes in the sample, the wells were washed to remove non-binding material. A labeled anti-human IL-1 α secondary Ab was then added to the wells (0.2 μ g/ml biotin-conjugated monoclonal mouse anti-human IL-1A Ab, clone CRM6, eBioscience catalog #13-7017). After allowing time to bind the IL-1 α in the wells, the plate was washed and the amount of labeled anti-human IL-1 α in each well was quantified as an indication of the concentration of IL-1 α in the sample being tested.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 12

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<211> LENGTH: 471

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 1

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Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
20     25     30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
35     40     45
Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50     55     60
Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65     70     75     80
Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
85     90     95
Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
100    105    110
Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
115    120    125
Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
130    135    140
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145    150    155    160
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165    170    175
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180    185    190
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195    200    205
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
210    215    220
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
225    230    235    240
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
245    250    255
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
260    265    270
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
275    280    285
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
290    295    300
Gly Val Glu Val His Asn Ala Gln Thr Lys Pro Arg Glu Glu Gln Tyr
305    310    315    320
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
325    330    335
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
340    345    350
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
355    360    365

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Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 370 375 380
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 385 390 395 400
 Ile Ala Leu Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 405 410 415
 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 420 425 430
 Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
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 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 450 455 460
 Leu Ser Leu Ser Pro Gly Lys
 465 470

<210> SEQ ID NO 2
 <211> LENGTH: 1416
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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gtgcagctgg tggagagtgg gggtagcgtg gtgcagcctg gccgggtctct gcgcctgtct      120
tgcactgcct ccggttttac cttttctatg tttggtgtgc actgggtgcg ccaggctccc      180
ggcaagggac tggaatgggt ggccgcctg agttacgacg ggtccaacaa atattacgct      240
gagagcgtga aaggcagatt caccatcagc agagataatt ccaagaatat tctgttctctg      300
cagatggaca gtctgagact ggaggacact gctgtgtact actgcgctcg tggacgccct      360
aagggtggtea tccccgcctc cctggcacat tggggccagg gaactctggt gaccttttct      420
agcgctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct      480
gggggcacag cggccctggg ctgcctggtc aaggactact tcccgaacc ggtgacgggtg      540
tcgtggaact caggcgccct gaccagcggc gtccacacct tcccggtgt cctacagtcc      600
tcaggactct actccctcag cagcgtagtg accgtgccct ccagcagctt gggcaccacg      660
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag      720
cccaaattct gtgacaaaac tcacacatgc ccacgtgcc cagcacctga actcctgggg      780
ggaccgtcag tcttctctct ccccccaaaa cccaaggaca ccctcatgat ctcccgacc      840
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac      900
tggtacgtgg acggcgtgga ggtgcataat gccagacaa agccgcggga ggagcagtac      960
aacagcacgt accgtgtggt cagcgtcttc accgtctctc accaggactg gctgaatggc     1020
aaggagtaca agtgcaaggt ctccaacaaa gccctcccag ccccatcga gaaaaccatc     1080
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atcccgggat     1140
gagctgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta tcccagcgac     1200
atcgccctgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1260
gtgtggact ccgacggctc cttcttctc tacagcaagc tcaccgtgga caagagcagg     1320
tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggctctgca caaccactac     1380
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<210> SEQ ID NO 3

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<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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20          25          30
Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35          40          45
Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
50          55          60
Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
65          70          75          80
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
85          90          95
Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100         105         110
Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
115         120         125
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
130         135         140
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145         150         155         160
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
165         170         175
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
180         185         190
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
195         200         205
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210         215         220
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225         230         235

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<210> SEQ ID NO 4
<211> LENGTH: 708
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4
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gtgacaatca cctgccgcgc ctctcaggga atctctagtt ggctggcctg gtaccagcag 180
aagcctggaa agggcccca gctgctgata tatgaagcct ccaacctgga gaccggcgtg 240
ccctctcgct tcagcggctc aggtcaggc agtgatttta ctctgacct cagctccctg 300
cagccagagg atttcgtac ttactactgc cagcagacct ctctctct gctgtccttc 360
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tatcccagag agggccaaagt acagtgaag gtggataacg ccctccaatc gggtaactcc 540
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcacccctg 600

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acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag 660
ggcctgagtt caccggtgac aaagagcttc aacaggggag agtggttag 708
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<210> SEQ ID NO 5
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 5
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Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20          25          30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
 35          40          45
Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50          55          60
Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
 65          70          75          80
Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
 85          90          95
Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
 100         105         110
Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
 115         120         125
Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
 130         135         140
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 145         150         155         160
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 165         170         175
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 180         185         190
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 195         200         205
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 210         215         220
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
 225         230         235         240
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 245         250         255
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 260         265         270
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 275         280         285
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 290         295         300
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 305         310         315         320
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 325         330         335
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 340         345         350
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
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355	360	365
Glu Pro Gln Val Tyr Thr	Leu Pro Pro Ser Arg	Asp Glu Leu Thr Lys
370	375	380
Asn Gln Val Ser Leu Thr	Cys Leu Val Lys Gly	Phe Tyr Pro Ser Asp
385	390	395
Ile Ala Val Glu Trp Glu	Ser Asn Gly Gln Pro	Glu Asn Asn Tyr Lys
405	410	415
Thr Thr Pro Pro Val Leu	Asp Ser Asp Gly Ser	Phe Phe Leu Tyr Ser
420	425	430
Lys Leu Thr Val Asp Lys	Ser Arg Trp Gln Gln	Gly Asn Val Phe Ser
435	440	445
Cys Ser Val Met His Glu	Ala Leu His Asn His	Tyr Thr Gln Lys Ser
450	455	460
Leu Ser Leu Ser Pro Gly	Lys	
465	470	

<210> SEQ ID NO 6

<211> LENGTH: 1422

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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tgccagggtcc agctgggtgga gtctggtggg ggagtgggtgc agcctgggag atctctgcgg    120
ctgtcttgca ctgcctctgg ttctacttct tctatgtttg gtgtgcattg ggtcaggcaa    180
gcaccaggga aaggactcga gtgggtcgca gctgtgagct atgacgggtc taacaaatat    240
tacgctgagt ctgtcaaggg taggtttacc atcagccggg ataattccaa aaatatcctg    300
ttcctgcaaa tggactctct gaggtgggaa gatactgcag tctactattg tgcaaggggg    360
aggccaaagg tggatgctcc cgtccccc cgtcactggg gacaggggaa cctgggtgact    420
ttcagctctg ctagcaccaa gggccctagc gtgttcccat tggctccttc ctccaaatct    480
actttctggg gcaccgcgcg cctgggatgt ctctgaaaag attattttcc tgagcccgct    540
accgtgagct ggaacagcgg cgcctctgact agcggcgtgc acacctttcc cgcagtgtct    600
caatctagcg ggctgtactc cctgagctct gtcgtgaccg tgccctccag cagcctcgga    660
actcagacct acatctgcaa tgtcaatcat aaacctcta ataccaaagt cgataagaag    720
gtcgaacctc aatcttgca taaaacctat acctgcccc cttgccagc acccgaaactg    780
ctgggcgggc cctctgtgtt tctgttcccc cccaaaccca aagataacct gatgatctct    840
aggacccccg aggtcacttg tgtcgtgggt gatgtgtccc acgaagatcc agaagtcaaa    900
ttcaactggg atgtggacgg ggtcgaagt cacaacgcaa agaccaagcc tagggaggaa    960
cagtataata gcacatatag ggtggtcagc gtcctgaccg tcttgcacaa ggactggctg   1020
aatggcaaag aatataagt taaagtgtcc aacaaggccc tgccagcccc aatcgaaaag   1080
acaatctcta aagccaaggg gcaaccccgg gaacctcagg tctatacact gccacctct   1140
cgggatgaac tgaccaagaa tcaggtgagc ctgacatgtc ttgtgaaggg tttttatccc   1200
tccgacattg ccgtggagtg ggagagcaat ggacaaccag aaaataacta caaaaccaca   1260
ccccctgtgc tggactccga tggttccttc ttcctctact ctaagctgac agtggataag   1320
tctaggtggc agcaggggaa tgtgttctcc tgctctgtga tgcacgaggc actgcacaat   1380
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<210> SEQ ID NO 7
 <211> LENGTH: 234
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

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20        25        30
Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35        40        45
Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
50        55        60
Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
65        70        75        80
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
85        90        95
Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100       105       110
Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
115       120       125
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130       135       140
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145       150       155       160
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165       170       175
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180       185       190
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195       200       205
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210       215       220
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225       230

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<210> SEQ ID NO 8
 <211> LENGTH: 711
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

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gacaggggtca caatcacttg tagggccagc caggggatct ctagttaggt cgcattggtac    180
caacaaaagc caggttaaggc tccgaaactg ctcatctacg aagctagtaa cctcgaaaca    240
ggcgtgccaa gccgggttag cggtccgggt tccggttctg acttcacct cactattttc    300
tccctgcaac ctgaggattt tgccacatat tactgtcagc aaactttctt ttttctgctc    360
tcctttgggt ggggaactaa ggtggagcac acagtggccg cccccagcgt ctttatcttc    420
cccccaagcg atgaacagct gaagtcaggg accgccagcg tggctgtgct gctcaataat    480
ttttaccctc gcgaggctaa ggtccaatgg aaagtggata acgcccctca gagcggtaac    540

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tctcaggagt ctgtcacaga gcaagacagc aaggatagca cctattccct ctccagcacc 600
ctgacactgt ctaaggccga ctacgagaaa cacaaagtgt acgcttgtga ggtgactcac 660
cagggactga gtagccctgt gacaaaatct ttcaataggg gagaatgctg a 711
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<210> SEQ ID NO 9
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 9
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Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
20     25     30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe
35     40     45
Ser Met Phe Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50     55     60
Glu Trp Val Ala Ala Val Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65     70     75     80
Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
85     90     95
Ile Leu Phe Leu Gln Met Asp Ser Leu Arg Leu Glu Asp Thr Ala Val
100    105    110
Tyr Tyr Cys Ala Arg Gly Arg Pro Lys Val Val Ile Pro Ala Pro Leu
115    120    125
Ala His Trp Gly Gln Gly Thr Leu Val Thr Phe Ser Ser Ala Ser Thr
130    135    140
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145    150    155    160
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165    170    175
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180    185    190
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195    200    205
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
210    215    220
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
225    230    235    240
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
245    250    255
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
260    265    270
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
275    280    285
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
290    295    300
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305    310    315    320
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
325    330    335
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
340    345    350
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Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 355 360 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
 370 375 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 385 390 395 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 405 410 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 420 425 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 435 440 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 450 455 460

Leu Ser Leu Ser Pro Gly Lys
 465 470

<210> SEQ ID NO 10

<211> LENGTH: 1422

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

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tgccaggtec agctgggtga gtctggtggg ggagtgggtc agcctgggag atctctgcgg      120
ctgtcttgca ctgcctctgg ttctacttct tctatgtttg gtgtgcattg ggtcaggcaa      180
gcaccaggca aaggactcga gtgggtcgca gctgtgagct atgacgggtc taacaaatat      240
tacgtctgagt ctgtcaaggg taggtttacc atcagccggg ataattccaa aaatatcctg      300
ttctcgcaaa tggactctct gaggtcgga gatactgcag tctactattg tgcaaggggg      360
aggccaaagg tggatgatcc cgctccctc gctcactggg gacagggaac cctggtgact      420
ttcagctctg ctagcaccaa gggccctagc gtgttcccat tggctccttc ctccaaatct      480
actttctggg gcaccgcgcg cctgggatgt ctctgtaaag attattttcc tgagcccgtc      540
accgtgagct ggaacagcgg cgccctgact agcggcgtgc acacctttcc cgcagtgtg      600
caatctagcg ggctgtactc cctgagctct gtctgacccg tgccctccag cagcctcgga      660
actcagacct acatctgcga tgtcaatcat aaacctcta ataccaaagt cgataagagg      720
gtcgaaaccta aatcttgcca taaaacccat acctgcccc cttgccccag acccgaaactg      780
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aggacccccg aggtcacttg tgtcgtggtg gatgtgtccc acgaagatcc agaagtcaaa      900
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tccgacattg ccgtggagtg ggagagcaat ggacaaccag aaaataacta caaaaccaca     1260
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<210> SEQ ID NO 11
 <211> LENGTH: 236
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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 Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
 35 40 45
 Gln Gly Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
 50 55 60
 Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn Leu Glu Thr Gly Val
 65 70 75 80
 Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr Leu Thr
 85 90 95
 Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
 100 105 110
 Thr Ser Ser Phe Leu Leu Ser Phe Gly Gly Gly Thr Lys Val Glu His
 115 120 125
 Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 130 135 140
 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 145 150 155 160
 Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 165 170 175
 Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 180 185 190
 Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 195 200 205
 Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
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<210> SEQ ID NO 12
 <211> LENGTH: 717
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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 gacaggggtca caatcacttg tagggccagc caggggatct ctagttaggt cgcattggtac 180
 caacaaaagc caggttaaggc tccgaaactg ctcatattacg aagctagtaa cctcgaaaca 240
 ggcgtgccaa gccggttttag cggtccgggt tccggttctg acttcacct cactatttcc 300
 tccctgcaac ctgaggattt tgccacatat tactgtcagc aaacttcttc ttttctgctc 360
 tcctttgggtg gaggaactaa ggtggagcac aagcggacag ttgctgctcc tagcgtcttt 420
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agcaccctga cactgtctaa ggccgactac gagaaacaca aagtgtacgc ttgtgaggtg	660
actcaccagg gactgagtag ccctgtgaca aaatctttca ataggggaga atgctga	717

What is claimed is:

10

1. A human IgG1 monoclonal antibody that specifically binds to interleukin-1 alpha (IL-1 α), wherein the monoclonal antibody comprises the amino acid sequence of an immunoglobulin produced by expressing in a mammalian host cell a first nucleic acid encoding the amino acid sequence of SEQ ID NO: 9 and a second nucleic acid encoding the amino acid sequence of SEQ ID NO: 11.

15

2. The human IgG1 monoclonal antibody of claim 1, wherein the mammalian host cell is a Chinese hamster ovary cell.

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